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Supplemental Data

Temporal Regulation of Metamorphic Processes in *Drosophila* by the *let-7* and *miR-125* Heterochronic MicroRNAs

Elizabeth E. Caygill and Laura A. Johnston

Supplemental References

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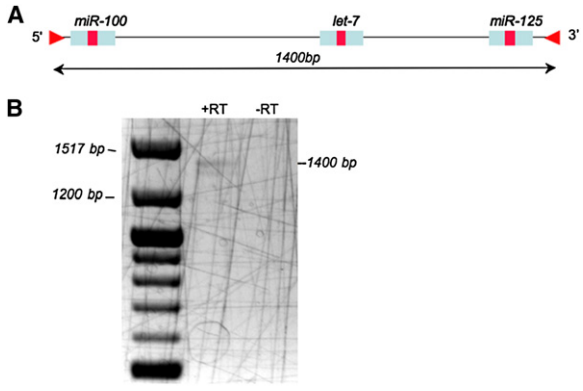

Figure S1. *miR-100*, *let-7*, and *miR-125* Are Expressed in a Single Polyadenylated Transcript

(A and B) The wild-type *let-7*, *miR-125* locus. Red arrowheads indicate positions of primer set three ([A], see Experimental Procedures), which flanks the three microRNAs and amplifies a 1400 bp product from poly(A)$^+$ RNA generated from wild-type pupae at 42 hr APF (B), confirming the existence of a single polyadenylated transcript containing all three miRNAs.

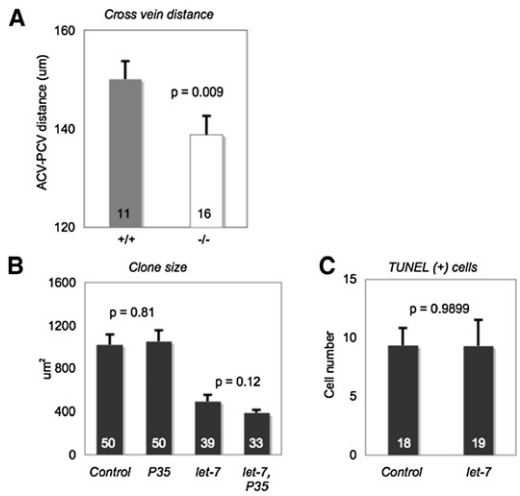

Figure S2. Wing-Phenotype Supportive Data

(A) Temporal defects in *let-7, miR-125* wing development. At 28 hr APF, the separation between the anterior and posterior crossveins is delayed in *let-7, miR-125* mutants (−/−) compared to wild-type (+/+). Numbers indicate number of wing discs analyzed.

(B) Inhibition of cell death does not suppress the small clone size due to misexpression of UAS-*let-7*. Shown is the size of Flp-out clones, which each express UAS-GFP, with or without expression of UAS-P35, the caspase inhibitor. Experimental clones express UAS-*let-7* or UAS-*let-7* + UAS-P35, as indicated. Blocking cell death with UAS-P35 did not alter clone size. Numbers on bars indicate number of clones examined.

(C) Misexpression of *let-7* does not increase death of wing disc cells. Graph shows the number of TUNEL-positive cells in wandering-stage larval wing discs containing GFP-marked control clones or clones expressing UAS-*let-7*. Numbers on bars represent discs examined. Error bars indicate standard error.

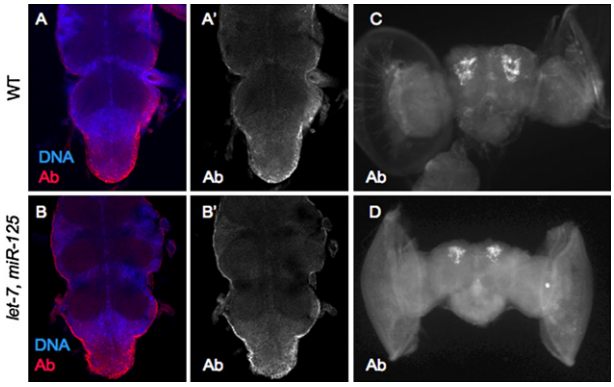

Figure S3. Ab Is Not Expressed in Motoneurons of the Abdominal Ganglion

(A and B) wild-type (A and A′) and mutant (B and B′) abdominal ganglia of the CNS stained for Ab and DNA. No specific Ab staining is detected in motoneurons. As a control for nuclear Ab staining, the brains of wild-type (C) and *let-7, miR-125* mutants (D) are shown; note that Ab is neither downregulated in wild-type cells in the brain nor affected by loss of *let-7* and *miR-125*.

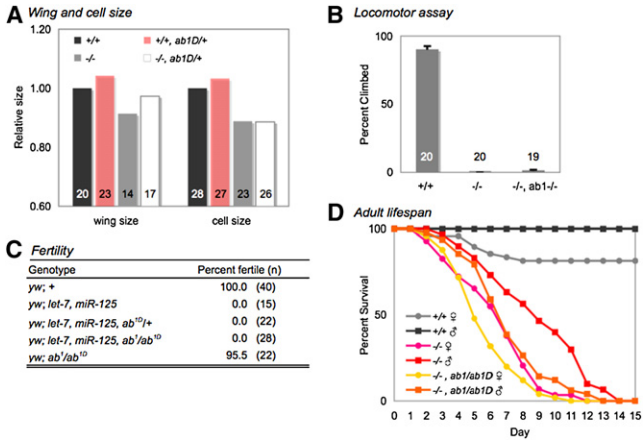

Figure S4. Reduction of *abrupt* Gene Dose Does Not Suppress All *let-7, miR-125* Defects

(A) The small wing and cell sizes of *let-7, miR-125* mutants are not rescued in the ab^{1D}/+ background. Reducing the dose of *ab* with the strong allele ab^{1D} did not alter the small cell size of *let-7, miR-125* mutant wings. Wing size was increased in *let-7, miR-125,* ab^{1D}/+ mutant wings; however, ab^{1D}/+ also produced a dominant increase in wild-type wing size. The number of wings examined for each genotype is noted on the bars.

(B) Defects in locomotion in *let-7, miR-125* mutants are not rescued by ab^{1}/+. The climbing response of wild-type controls and *let-7, miR-125* mutants to a tap was monitored. The response of mutant files was severely diminished and was not suppressed by the presence of the ab^{1} allele. Data from females are shown; males performed similarly. Note that ab^{1} is a weak allele of *ab*.

(C) Fertility is not restored in *let-7, miR-125, ab* double mutants. Reducing the dose of *ab* in *let- 7, miR-125* mutants with the strong allele ab^{1D} alone or in a transheterozygous combination with the weak allele ab^{1} does not restore fertility.

(D) Adult lifespan, which is significantly reduced in male and female mutants, is not rescued by the ab^{1}/ab^{1D} genetic background. Wild-type (+/+) females, n = 49; males, n = 50; *let-7, miR-125* (−/−) females, n = 29; males, n = 30; −/−, ab^{1}/ab^{1D} females, n = 50; males, n = 49.

Table S1. Phenotype of *let-7* or *miR-125* Misexpression

Gal 4 Driver	Expression Pattern	Timing[a]	UAS-*let-7*	UAS-*miR-125*
tub Gal 4	Ubiquitous	E, L, P	Larval lethal (L1/L2)	Larval lethal (L1/L2)
en Gal4	All posterior cells	E, L, P	Larval lethal (L1/L2)	ND
dpp Gal 4	Anterior, imaginal discs	L, P[b]	Pupal lethal[d]	Pupal lethal[e]
vg Gal 4	Wing imaginal disc	L, P[b]	Viable, small wings	viable
A ct5C > Gal4	Clonal	L[c]	Viable, cell-cycle arrest	ND

[a] Expression during E = embryonic, L = larval, and P = pupal stages.
[b] Mid-second instar.
[c] Clones induced early third instar.
[d] Predifferentiation.
[e] Postdifferentiation.

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Table S2. Tissue-Specific Misexpression of *abrupt*

Gal 4 Driver	Expression	UAS-*abrupt*
MEF2	Muscle	Larval lethal (L3)
24B	Muscle	Larval lethal (L2)
C155	Pan neuronal	Eclose
OK6	Motoneuron	Eclose
CHA	CNS (Ach+ neurons)	Larval lethal (L3)

Gal 4 drivers (column 1) were used to express UAS-*abrupt* in the tissues indicated (column 2). The resulting phenotype is listed in column 3. References for each driver are as follows: MEF2 Gal 4 [S1], 24B Gal 4 [S2], C155 Gal 4 [S3], OK6 Gal 4 [S4], and CHA Gal 4 [S5].